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BRAIN RESEARCH

Research Report

Effects of chloride flux modulators in an in vitro model of brain edema formation

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ARTICLE INFO

Article history:
Accepted 6 September 2006
Available online 2 October 2006

Keywords:
Edema
Furosemide
DIDS
Niflumic acid
NMDA receptor
Oxygen–glucose deprivation

Abbreviations:
DIDS,
4,4′-diisothiocyanostilbene-2,2′-disulfonic acid
NMDA,
N-methyl-D-aspartate
OGD,
oxygen–glucose deprivation

ABSTRACT

Brain edema is a serious consequence of hemispheric stroke and traumatic brain injury and contributes significantly to patient mortality. In the present study, we measured water contents in hippocampal slices as an in vitro model of edema formation. Excitotoxic conditions induced by *N*-methyl-D-aspartate (NMDA, 300 μM), as well as ischemia induced by oxygen–glucose deprivation (OGD), caused cellular edema formation as indicated by an increase of slice water contents. In the presence of furosemide, an inhibitor of the Na,K,Cl-cotransporter, NMDA-induced edema were reduced by 64% while OGD-induced edema were unaffected. The same observation, i.e., reduction of excitotoxic edema formation but no effect on ischemia-induced edema, was made with chloride transport inhibitors such as DIDS and niflumic acid. Under ischemic conditions, modulation of $GABA_A$ receptors by bicuculline, a GABA antagonist, or by diazepam, a GABAergic agonist, did not significantly affect edema formation. Further experiments demonstrated that low chloride conditions prevented NMDA-induced, but not OGD-induced, water influx. Omission of calcium ions had no effect. Our results show that NMDA-induced edema formation is highly dependent on chloride influx as it was prevented by low-chloride conditions and by various compounds that interfere with chloride influx. In contrast, OGD-induced edema observed in brain slices was not affected by modulators of chloride fluxes. The results are discussed with reference to ionic changes occurring during tissue ischemia.

1. Introduction

Brain edema formation, which is observed after large hemispheric stroke or traumatic brain injury, is one of the most dangerous consequences of acute ischemia and excitotoxicity in the brain (Steiner et al., 2001; Unterberg et al., 2004). While water contents in healthy brains are closely regulated by a variety of homeostatic mechanisms (Kimelberg, 2004), breakdown of these mechanisms in ischemia, including severe dysregulations of ionic distributions, causes swelling of the brain and increased intracranial pressure which is one of the premier factors determining survival in patients (Reilly, 2001). Early brain swelling is known to be due to cellular ("cytotoxic") edema while vasogenic edema develop in a more delayed fashion (over hours). Among the brain regions, the hippocampus has been found to be highly susceptible to ischemia;

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doi:10.1016/j.brainres.2006.09.012

pyramidal cells of the CA1 region are most sensitive (Schmidt-Kastner and Freund, 1991). Astrocytes are well known to undergo swelling when excitotoxic concentrations of glutamate are present (Kimelberg, 2005; Seifert et al., 2006), and they contribute strongly to ensuing changes of intracranial pressure. Inward transport of sodium, calcium, chloride and water is known to cause edema and subsequent toxicity in neurons; potassium uptake additionally contributes to astrocytic swelling (Hansen, 1985; Somjen, 2002).

Hippocampal slices have recently been introduced as an *in vitro* model of brain edema formation induced by ischemia (MacGregor et al., 2003). Water contents of slices can be determined by a simple differential weighing procedure and, with short incubation times of less than one hour, reflect cellular edema formation. Preparation and superfusion of hippocampal slices *per se* already causes a minor swelling of slices over 30–60 min, which is accompanied by sodium and calcium uptake (Siklos et al., 1997). During in vitro ischemia (oxygen–glucose deprivation, OGD), an increase of sodium and calcium uptake was observed to which voltage-operated cation channels as well as glutamate receptors of the AMPA and NMDA subtypes contributed (LoPachin et al., 2001; MacGregor et al., 2003). Inhibitors of AMPA and NMDA cation channels, as well as sodium channel blockers and antioxidants, were found to attenuate edema formation in earlier studies (LoPachin et al., 2001; MacGregor et al., 2003). The importance of sodium and calcium influx for ischemia-induced edema formation and cellular injury was also documented in organotypical slice cultures exposed to OGD (Breder et al., 2000; Martinez-Sanchez et al., 2004) and in neuronal cell cultures (Goldberg and Choi, 1993; Czyz et al., 2002).

Compared to the extensive data on the importance of cation movements (see above), the significance of anions such as chloride for brain edema formation has only recently drawn attention (Somjen, 2002; see Discussion for further references). In the present study, we investigated the relevance of chloride influx for edema formation. In addition to OGD, we also used *N*-methyl-D-aspartate (NMDA) as a stimulator of edema formation in hippocampal slices. NMDA receptors have a major role in ischemia-induced neurotoxicity (Lipton, 1999; Arundine and Tymianski, 2004); they not only depolarize neurons but allow influx of large amounts of calcium ions that are detrimental to the cells. Importantly, it is known that NMDA receptor-mediated neurotoxicity is dependent on extracellular chloride. Studies in neuronal cell cultures (Rothman, 1985; Olney et al., 1986; Choi, 1987) demonstrated that cellular influx of chloride ions is required for cytotoxicity induced by glutamate and NMDA. A small number of follow-up studies supported this hypothesis and have shown protective effects of chloride-free media against NMDA-induced toxicity in organotypic cultures and brain slices (Takahashi et al., 1995; Gröndahl et al., 1998). There are also studies that discuss beneficial or malignant effects of ischemia-induced chloride fluxes through ligand-operated chloride channels such as $GABA_A$ receptors (Erdö et al., 1991; Hasbani et al., 1998; Inglefield and Schwartz-Bloom, 1998; Chen et al., 1999; Galeffi et al., 2004; Babot et al., 2005). In the present study, we tested the effects of ionic manipulations, chloride transport inhibitors, and GABA modulators on NMDA- and ischemia-induced edema formation in hippocampal slices. Our results confirm previous reports on the chloride dependence of NMDA-induced responses but caution against an extrapolation of these findings to ischemia-induced edema.

2. Results

2.1. *In vitro model of brain edema formation*

Hippocampal slices that were superfused with control buffer for 30 min had average water contents of 77–81% (Figs. 1–4). Some of this variation was probably due to variations in preparation time, which was generally kept below 6 min from decapitation of the animals to superfusion of the slices. To mimic excitotoxicity, slices were exposed to NMDA (300 μM). In

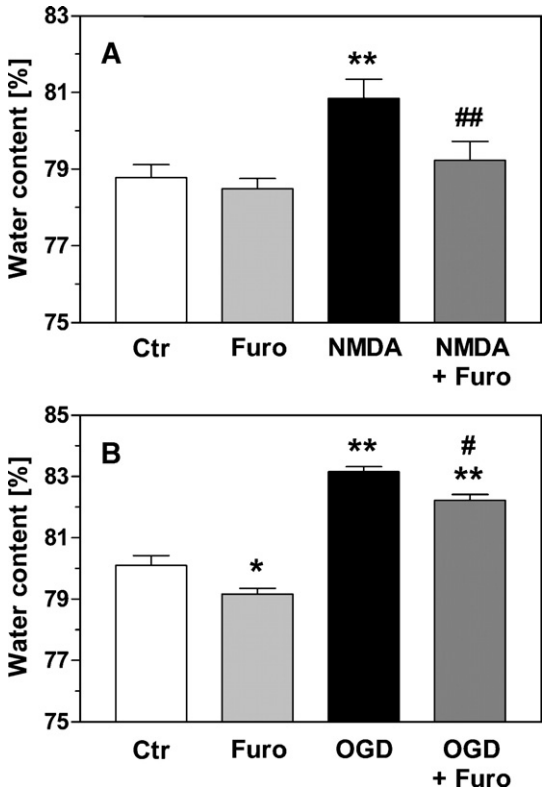

Fig. 1 – Tissue water contents in hippocampal slices: effects of furosemide. (A) Slices were superfused under control conditions ("Ctr") or with furosemide ("Furo", 10 μM). Edema was induced by *N*-methyl-D-aspartate in the absence ("NMDA", 300 μM) or presence ("NMDA + Furo") of furosemide ($N = 5$). (B) Slices were superfused under control conditions or with furosemide, as in (A). Edema was induced by oxygen–glucose deprivation ("OGD") in the absence ("OGD") or presence ("OGD + Furo") of furosemide ($N = 5$). Slices were exposed to NMDA or OGD for 30 min; when furosemide was present, it was added 5 min before OGD. All superfusions were done in the presence of 0.1% DMSO, which was used to dissolve furosemide. Water contents were determined at the end of the superfusion by a differential weighing procedure before and after drying the slices. Statistical significance was evaluated by paired ANOVA. $^{*}p < 0.05$; $^{**}p < 0.01$ vs. controls (Ctr). $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ vs. NMDA or OGD, respectively.